Tumour angiogenesis: **Hitting cancer where it hurts**

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Two recent studies that involve perturbing tumour blood supply provide new hope for anti-cancer therapies. The first uses elegant molecular engineering to achieve tumour-specific blood clots and the second reports the identification of a natural inhibitor, endostatin, which is produced from tumour extracellular matrix.

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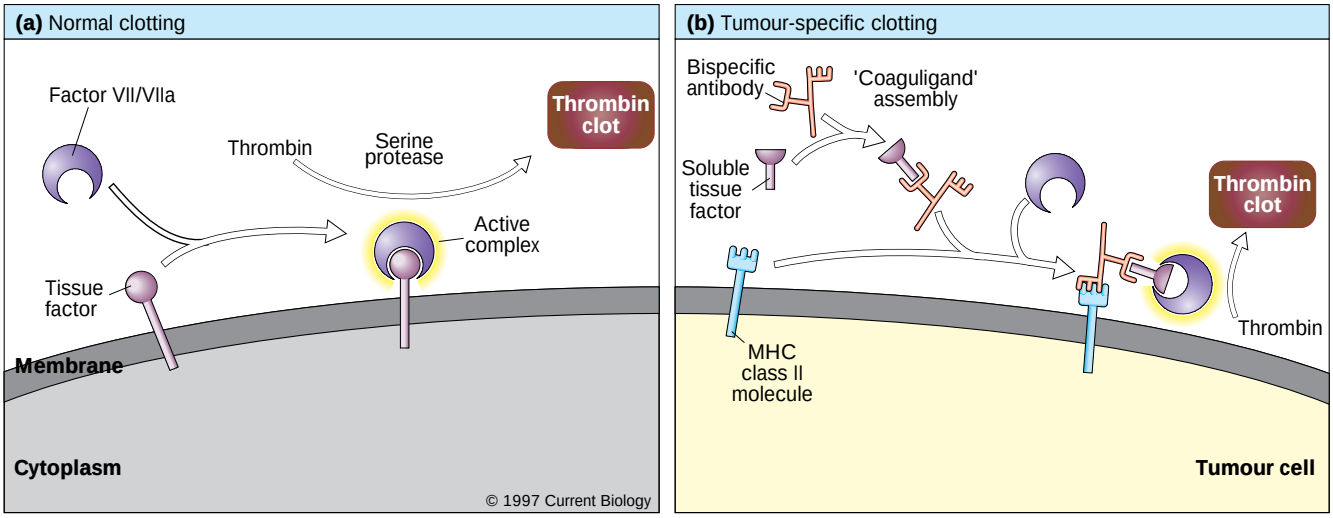
Tumorigenesis is a complex and multi-factorial process. In the first instance, it requires the accumulation of a series of genetic mutations within preneoplastic cells which uncouple the cells' normally highly interdependent programmes of growth and differentiation. But oncogenic mutations cannot liberate the expanding tumour population from the need to supply itself with nutrients and oxygen, which must come from a nearby blood supply. Thus, it has become clear that a growing tumour needs to stimulate not only its own cells' growth but also growth of infiltrating blood vessels, expansion of which is critical for the nourishment of the tumour. The process of stimulating growth of the vasculature is called angiogenesis, and its central significance for tumorigenesis is underscored by the large numbers of angiogenic factors that are now known to be produced by tumours [1]. Indeed, in the absence of angiogenesis, no tumour can grow larger than 1–2 mm in diameter, and the degree of vascularization of a tumour can be one of the most reliable prognostic indicators of clinical outcome in several tumour types [2].

The proven ability of tumours to supply their own nutritional requirements by promoting angiogenesis has depressing clinical implications — as it means that tumours can grow in size without outstripping their nutritional requirements. But the fact that tumours need to promote angiogenesis offers distinct opportunities for therapy. Tumours of many diverse histological types must induce angiogenesis to survive, and the angiogenic pathways used are, for the most part, independent of tumour type. So, whereas different tumours show widely differing susceptibilities to conventional anti-cancer therapies, such as chemotherapy and radiotherapy, an effective anti-angiogenic therapy might have wide-spectrum applicability as a general anti-cancer treatment.

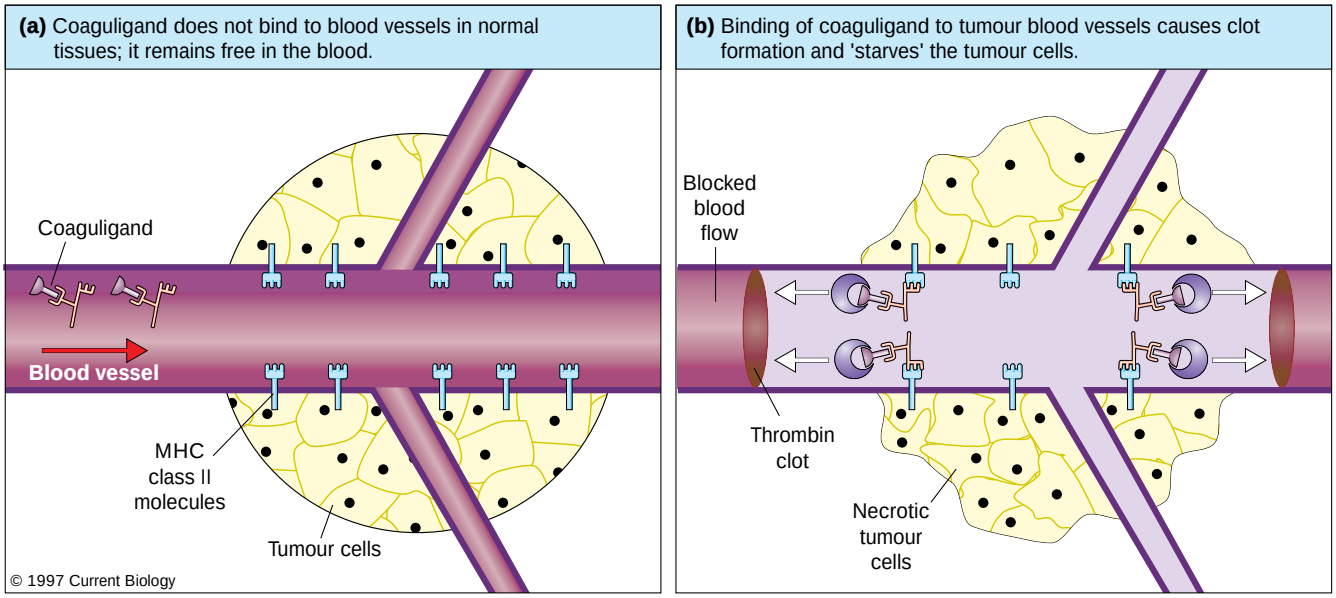
Attractive as they are as intervention therapies, anti-angiogenic strategies must still be developed which do not interfere pathologically with the normal angiogenic

Figure 1



Induction of a blood clot specifically in tumour blood vessels, as described by Huang *et al.* [3]. **(a)** Tissue factor is active only when part of a membrane-associated complex. **(b)** Soluble tissue factor has limited activity, unless it is anchored to the membrane by association with the bispecific antibody that also recognizes the MHC class II molecule expressed only on the surface of tumour endothelial cells.

Figure 2



The 'coaguligand' targets clotting to tumour blood vessels and so cuts off the tumour's blood supply.

requirements of the body. One elegant method by which tumour-specific angiogenesis might be therapeutically targeted has recently been described by Philip Thorpe's group [3]. These workers reasoned that an effective way to kill a tumour would be to induce a thrombosis (blood clot) at the tumour site. They focused on a key molecule involved in the coagulation process, tissue factor (TF), which initiates the blood clotting cascade. Normally, tissue factor associates with another clotting factor, Factor VII/VIIa, to activate serine proteases which themselves lead to thrombin production and the formation of a blood clot (Fig. 1a). Importantly, TF is fully active only as part of a molecular complex tethered within a phospholipid membrane. Huang and coworkers [3] constructed a truncated, soluble form of TF (sTF), which, because of its solubility, has minimal coagulation-inducing properties when administered free in the circulation. They linked the sTF to a bispecific antibody which recognizes both the sTF molecule and a second molecule which is present on the cell surface — in this case a class II molecule of the major histocompatibility complex (MHC; Fig. 1b). The group had previously shown that, when interferon-γ-expressing neuroblastoma cells are implanted *in vivo* as progressively growing tumours, the endothelial cells lining the blood vessels within these tumours showed greatly enhanced expression of the MHC class II molecule which, significantly, is absent from normal endothelial cells lining blood vessels in nontumour cells *in vivo*. So, the pieces were in place to provide an experimental model in which a targeting molecule — the antibody — with specificity for a marker known to be expressed specifically on the tumour blood vessel endothelium — the MHC class II molecule — was linked to a molecule which could induce clot formation when immobilized on a cell surface, namely sTF (Fig. 1).

When the cytokine-expressing neuroblastoma cells were implanted as tumours into mice which were then treated systemically with the antibody–sTF complex — which the authors named the 'coaguligand' — histologically observable thromboses were induced within the growing tumours and, by 72 hours after administration, the entire central region of the treated tumours was necrotic (Fig. 2). Animals that received tumour cells but no coaguligand showed no such anti-tumour effects. These histological observations also translated into therapeutic benefits: mice with large, established tumours underwent complete (38%) or substantial partial (50%) regressions when treated with coaguligand. Some small anti-tumour effects were also seen in mice treated with sTF alone, an effect attributed to the residual thrombogenic effects of the soluble molecule by itself. However, no thrombi were detectable in various other nontumour tissues in the mice treated with coaguligand or sTF, showing that this approach is potentially highly tumour-specific and, therefore, safe.

Clearly several problems have to be resolved before this system becomes therapeutically useful — not least the identification of surface markers which are expressed specifically on the endothelial cells of blood vessels supplying human tumours. Only then will it be possible to target soluble clotting factors to tumours whilst preserving the free flow of blood throughout other body sites. In this

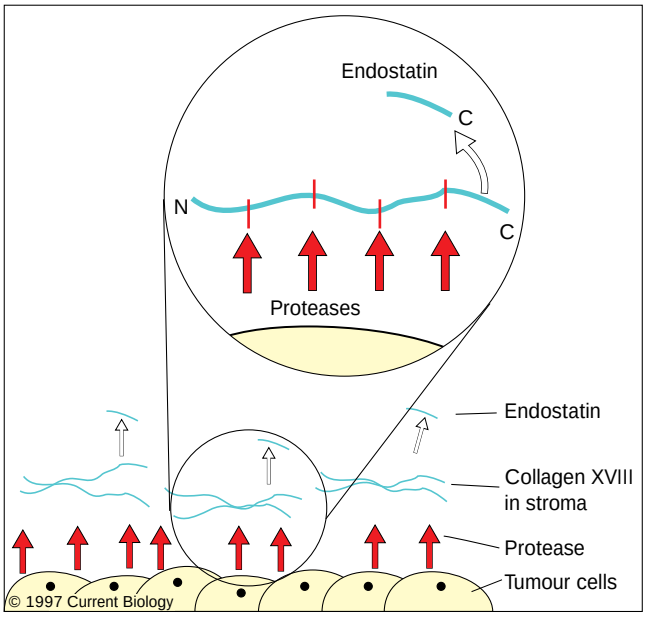
respect, it is worth noting that tumour endothelial cells are known to express several molecules, such as the receptor for vascular endothelial cell growth factor (VEGF), which are normally absent from quiescent endothelium and which might allow coaguligand-mediated clot targeting.

A second recent report which gives hope for effective anti-angiogenic targeting comes from Judah Folkman's laboratory [4] and has an air of *deja vu* about it. Two years ago, the elegant and dramatic discovery of angiostatin from Folkman's laboratory [5] was reported in these pages [6]. Angiostatin, a 342 amino-acid peptide, is an internal proteolytic fragment of plasminogen, is strongly anti-angiogenic *in vitro*, and can suppress the growth of Lewis lung carcinoma metastases in tumour-bearing mice [5]. One of the many fascinating, but unanswered, questions raised by its discovery was whether angiostatin — and particularly the way in which it is produced from plasminogen *in vivo* — is one of a kind, or whether it heralded a new, general phenomenon whereby molecules could be readily generated from pre-existing, larger precursors that have completely separate activities. Now, with the description of a novel molecule christened endostatin, it seems that we have the answer.

Endostatin emerged from a screen of material, released from a hemangioendothelioma cell line, which could inhibit the proliferation of endothelial cells *in vitro*. The biological activity, which was clearly separate from angiostatin, was purified to homogeneity as a 20 kDa protein. Microsequencing of the protein revealed identity to an internal fragment of collagen XVIII (Fig. 3). The coding sequence for the peptide fragment was expressed in both bacterial and insect cell expression systems and the baculovirus-expressed endostatin behaved very similarly to the 20 kDa activity purified from the cell line and was a very effective inhibitor of angiogenesis *in vitro* [4].

Interestingly, on purification, the bacterially expressed protein was insoluble, but it could move slowly into solution and was effective in the anti-angiogenic assay. Although this property was initially problematic for testing, Folkman's group used it to provide a slow-release "biodepot" of endostatin, by implanting the insoluble protein *in vivo* and allowing it to solubilize slowly. With this method of drug delivery, endostatin was able to inhibit the growth of both primary tumours and metastases of lung carcinoma cells *in vivo* and, remarkably, well- established tumours of several histological types could be induced to regress as long as endostatin therapy was maintained. The induction of tumour regression and chronic dormancy is very reminiscent of the properties of angiostatin [5,7]. These results offer promise of a kind rarely seen in cancer therapeutics — activity against a broad spectrum of primary tumour types combined with the ability to cause shrinkage of large, established growths.

Figure 3



Endostatin is released by matrix-associated proteases from the carboxyl terminus of collagen XVIII in the stroma surrounding tumour cells.

Although the properties of angiostatin and endostatin are phenotypically very similar, there is no clear indication yet as to whether they exert their effects in the same way biochemically. This is not a trivial point. The history of anticancer therapeutics has been plagued by the discovery of active compounds followed by the development of tumour populations resistant to them, which led to the paradigm of combination therapies for cancer. The discovery of a molecular companion for angiostatin offers the chance of using combinations of anti-angiogenic therapies. Thus, if a tumour is found to be resistant to angiostatin it may be that it is sensitive to endostatin — or *vice versa*. Similarly, tumours which produce a certain spectrum of proteases may not be able to release angiostatin from its precursor molecule plasminogen; however, the same tumour may produce the proteases required to liberate endostatin from collagen XVIII. The identification of the biochemical mechanisms used by these molecules to inhibit endothelial cell proliferation, and the possibilities of their use in combination, will be keenly awaited.

Given the similarities of the properties of endostatin and angiostatin, the real significance of the new *Cell* paper [4] is not so much what endostatin does but where it comes from. The genealogy of angiostatin and endostatin suggests that multiple extra layers of biological information can be stored in the tertiary structure of pre-existing proteins, and that proteolysis may be a mechanism by which that information can be retrieved (Fig. 3). Although proteolytic cleavage has long been known to be a way that latent activities

of enzymes can be activated, these 'pro-proteins' have usually been recognized only as precursors of the active species. In contrast, here we have molecules such as plasminogen and collagen XVIII, which are already serving defined roles, from which can be released internal fragments with completely separate activities. As with many of the most interesting studies, these findings raise many further questions. How many more molecules contain extra functions within their three-dimensional structures? Can internal fragments of this type have activities other than as angiogenesis inhibitors? Are the activities of angiostatin and endostatin relevant *in vivo* in the context in which they have been found, or do they have other roles that have not yet been identified because of the nature of the assays used to date?

Given the extensive ends to which tumours have gone to ensure a continued, and expanding, supply of blood, the results of O'Reilly *et al.* [4] are surprising in that they suggest that tumours are simultaneously shooting themselves in the foot. Tumour cell invasion, leading to metastasis, is promoted by the secretion of various proteases by expanding tumours [8]. These proteases digest the stroma around the tumour, allowing expansion of the cells into the surrounding areas and, eventually, into the blood stream for metastatic dispersal [9]. Recently, angiostatin has been shown to be produced by the cleavage of plasminogen by a serine protease released from (prostate) tumour cell lines [10]. This raises the intriguing prospect that the tumour cells themselves secrete proteases which, on the one hand, promote their malignant potential whilst simultaneously checking their angiogenic and proliferative capacities.

What could be the evolutionary origins of this pathway, in which it appears that the body is fighting back against its malignant foe? It is tempting (and somewhat comforting) to speculate that here is a very elegant evolutionary twist, in which biology has devised a way to use the tumour's aggressiveness (secretion of proteases) against itself. Alternatively, the significance of these molecules in the control of tumorigenesis may be purely coincidental and their true function remains to be identified.

As elegant and effective as the coaguligand study of Huang and colleagues [3] is, the discovery of angiostatin [5], and now endostatin [4], seems to have more immediate clinical applicability. Both the coaguligand and endostatin can shrink large animal tumours but, unlike the targeted clotting approach, endostatin is naturally derived from tumours without the need for molecular manipulations. In addition, it maybe that the angiostatin and endostatin examples are showing us that we should be searching for a whole new stratum of biological information in which the 'first degree' tertiary structure of a protein may be further divisible into (multiple) 'second degree' tertiary structures that have completely separate associated biological functions. These studies seem likely to prove major contributions to understanding both potential cancer therapies and fundamental physiology.

References

1. Folkman J, Shing Y: Angiogenesis. *J Biol Chem* 1992, 267:10931–10934.
2. Weidner N, Semple JP, Welch WR, Folkman J: Tumour angiogenesis and metastasis-correlation in invasive breast carcinoma. *N Engl J Med* 1991, 324:1–8.
3. Huang X, Molema G, King S, Watkins L, Edgington TS, Thorpe PE: Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature. *Science* 1997, 275:547–550.
4. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997, 88:277–285.
5. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal R, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 1994, 79:315–328.
6. Vile RG: Cancer therapy: less blood means more more sanguinity. *Curr Biol* 1995, 5:10–13.
7. O'Reilly MS, Holmgren L, Chen C, Folkman J: Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nat Med* 1996, 2:689–692.
8. Vile RG: Invasion in cancer metastasis – the journey from the primary tumour to the circulation. In *Cancer Metastasis: from Mechanisms to Therapies*. Chichester, UK: John Wiley; 1995.
9. Hart IR, Saini A: Biology of tumour metastasis. *Lancet* 1992, 339:1453–1457.
10. Gately S, Twardowski P, Stack MS, Patrick M, Bogglo L, Cundiff DL, Schnaper HW, Madison L, Volpert O, Bouck N, *et al.*: Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor angiostatin. *Cancer Res* 1996, 56:4887–4990.